HISTOLOGICAL STUDY ON THE EVOLUTION OF THE PERIPHERAL NERVOUS SYSTEM IN RABBITS

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by

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I. INTRODUCTION

In all vertebrates the nervous system is derived exclusively from the ectoderm. The neural plate which gives rise to the greater part of the nervous system becomes differentiated on the dorsal surface of the embryo in the early stage of ontogeny. The central nervous system develops very rapidly in the early stages of vertebrate embryos and soon becomes definitely outlined. The further development of the peripheral nervous system of the vertebrates advances from the central nervous organ toward the periphery.

HELD (1909), HARRISON (1907-1910) and SPEIDEL (1933) proved that peripheral nerve fibers develop from the process of nerve cells. The SCHWANN's cells are known to be of ectodermal origin (HELD and HARRISON). WESTPHAL (1894) stated that the myelin sheaths of peripheral nerve fibers become mature in the sixth week after birth. According to KISS and MIHALIK (1930), however, the myelin sheaths appear during the fourth month in utero and are already mature at the time of birth. Except for these achievements, our knowledge of the conditions which obtain in the course of evolution of the peripheral nervous system is still very incomplete.

The author studied histologically the evolution of the peripheral nervous system in respect to the following points: 1) Development of the distribution of the peripheral nerve fibers. 2) Development of the "nervous syncytium" (JABONERO) and its relationship to that of the capillary system. 3) Development of nerve plexuses and of nerve cells in the wall of the intestinal canal. 4) Development of the myelin sheaths.

II. MATERIALS AND METHODS

Materials: Rabbits from fetuses to adults were classified in nine stages as follows. 1) Early fetal stage (within ten days of conception). 2) Middle fetal stage (from eleven to twenty days after conception). 3) Late fetal stage (from the twentieth to thirtieth days of pregnancy). 4) New-born. 5) 15th day after birth. 6) 1 month old. 7) 2 months old. 8) 3 months old. 9) Adult rabbits.

Method: Tissue fragments obtained from the ear, the small intestine and the hilum of the lung were fixed immediately after sacrifice in 10 per cent neutral

formalin solution for four months. Frozen sections from 30 to 40 microns thick were impregnated by the silver carbonate method of JAEONERO. And for staining the myelin sheath, EHRLICH's acid hematoxylin method was used.

III. HISTOLOGICAL FINDINGS IN THE PERIPHERAL NERVOUS SYSTEM IN EACH STAGE

1) Early fetal stage (about 1 cm long)

Thick bundles of nerve fibers run through undifferentiated tissues (Fig. 1). They may be interpreted as spinal nerves in respect to their distribution (Fig. 2). Fine nerve fibers arborize from these bundles and run irregularly into undifferentiated tissues (Fig. 3). Nervous syncytia and nuclei of SCHWANN's cells are not recognized. The myelin sheaths are not stained by EHRLICH's method. In the prevertebral ganglion (Fig. 4) nuclei of nerve cells are noticeably impregnated, though the cytoplasm of nerve cells is vague and accessory cells are not found (Fig. 5). Capillary veins are not recognized. But in the hilum of the lung there is a primordial tissue of large blood vessels with few thin nerve fibers (Fig. 6).

2) Middle fetal stage (about 3 cm long)

Nerve fibers arrive in the peripheral parts of the ear (Fig. 7) and have a tendency to form fine networks (Fig. 8). A few nuclei of SCHWANN's cells are found (Fig. 9). The myelin sheaths are not stained. The cytoplasm of nerve cells in the prevertebral ganglion is still vague, and accessory cells do not appear (Fig. 10). Capillary veins are rarely observed.

3) Late fetal stage (about 6 cm long)

The nerve fibers increase in number and spread out in the connective tissue of the ear (Fig. 11). The fine branches ramify in spaces between the primordium of hair follicles (Fig. 12). A few immature nuclei of SCHWANN's cells can be found along the nerve fibers (Fig. 13). In AUERBACH's plxus of the intestine, nuclei of nerve cells are relatively well impregnated, but their cytoplasm and accessory cells are hardly recognizable (Fig. 14). A thick network of nerve fibers in the muscle layer of the intestine (the 1st order according to REISER and STOEHR, jr.) is considerably developed, though fine networks (2nd and 3rd order, according to REISER and STOEHR, jr.) have not yet appeared (Fig. 15). Capillary veins are found here and there and some of them are accompanied by fine nerve fibers (Fig. 16).

4) New-born

Wavelike bundles of nerve fibers increase in number and in thickness in the root of the ear (Fig. 17). In the periphery of the ear fine networks of nerve fibers run toward the hair follicles which have just developed in considerable numbers (Fig. 18). A few nervous syncytia can be seen (Fig. 19), though the nuclei of SCHWANN's cells or interstitial cells (CAJAL) are still scanty (Fig. 20). The myelin sheaths are not stained by EHRLICH's method. The lst order of intramural nerve plexuses in the intestine becomes complicated and networks of the 2nd order appear slightly (Fig. 21 and 22). In the submucous tissue fine nerve fibers run towards

the mucous membrane (Fig. 23). The cytoplasm of nerve cells in the plexuses are slightly outlined, and accessory cells appear, but are still indistinct (Fig. 24). Capillary veins spread to some extent, but do not form networks.

5) Rabbits on the 15th day after birth

Nerve fibers in the subcutaneous tissue of the ear branch out intricately (Fig. 25) and have various thicknesses (Fig. 26). Some of them concentrate near the hair follicles which have fully developed (Fig. 27). Nervous syncytia increase in number and their cytoplasmic components are somewhat enriched (Fig. 28). First, the myelin sheath can be found (Fig. 29). The intramural distribution of nerve fibers in the intestine increases in general (Fig. 30). The cytoplasm of nerve cells is more clearly outlined than those of the new-born, and some of them are surrounded by accessory cells which increase in number (Fig. 31). In the submucous tissue, MEISSNER's plexus (Fig. 32) and nervous syncytia (Fig. 33) can be found. Capillary networks cannot be seen yet.

6) One month old rabbits

The number of nervous syncytia is greatly increased (Fig. 34) and the cytoplasmic components are also fully developed (Fig. 35). In this stage, capillary networks are fully developed (Fig. 36). The distribution of these capillary networks is more dense than that of adult rabbits. In the wall of the intestines, nerve cells and AUERBACH's accessory cells are remarkably clealy impregnated (Fig. 37). The nerve cells in MEISSNER's plexus, especially, are increased in number (Fig. 38). The 1st, 2nd and 3rd order of intramural nerve fibers become very complicated (Fig. 39). Myelin sheaths are definite. In this stage, as a whole, the development of various elements of the peripheral nervous system is in the process of completion.

7) Two months old rabbits

The concentrated ramifications of nerve fibers in the hair follicles are clearly observed (Fig. 40). On the other hand, the density of capillary networks is rather reduced and their distribution is similar to that of adult rabbits (Fig. 41). Nervous syncytia involving fibrous and cytoplasmic components are fully matured (Fig. 42 and 43). Also the intramural distribution of nerve fibers and the capillary supply in the intestinal canal are completely developed (Fig. 44). Nerve cells and accessory cells in both AUERBACH's and MEISSNER's plexuses are clearly outlined, and fine fibrillar meshes in the cytoplasm of nerve cells are abundant (Fig. 45). It is supposed that the nerve supply in the wall of the intestines at this stage is almost the same as in adult rabbits.

8) Three months old rabbits

In this stage the characteristics of the peripheral nervous system are very similar to those of the 2nd month. The nervous syncytia (Fig. 46), MEISSNER's plexus (Fig. 47) and the neurofibrillar distribution with nerve cells in the wall of the intestine (Fig. 48) are completely mature.

9) Adult rabbits

The characteristics of the peripheral nervous system in adult rabbits are the same as those of the 3rd month.

IV. SUMMARY AND CONSIDERATIONS

Embryologic studies on the nervous system have been reported by many authors. The majority studied the conditions especially in the very early stages of embryos. However, systematic studies of the evolution of the peripheral nervous system are few, except for those on the development of myelin sheaths by WESTPHAL (1894), KISS and v. MIHALIK (1930), SOKOLOUSKY (1931) and others. The author investigated the development of the peripheral nervous system of rabbits from fetuses to adults and obtained the following results.

1) The development of the nerve supply of the hair follicles.

Fine nerve fibers run through the tissues between the primordia of hair follicles which appears in the late fetal stage. In the ear of rabbits 15th days after birth, they have just arrived at hair follicles which have already developed. Two months after birth, they gradually develop and form concentrated ramifications around the hair follicles. The characteristics of nerve fibers in the ear of 2 months old rabbits are almost the same as those in adults. The specific nerve supply for the hair follicles begin within 15 days after birth and is almost completed in the 2nd month.

2) The development of nervous syncytia and its relationship to the evolution of the capillary system.

In the early fetal stage, nervous syncytia cannot be found. In the middle fetal stage, fine nerve fibers have a tendency to form a sort of meshwork without the appearance of cytoplasmic components such as SCHWANN's cells. In the late fetal stage, the nuclei of SCHWANN's cells are first observed, but very scant and vague. Thereafter nervous syncytia are gradually enriched and become fully mature in the 2nd month. That is, the first appearance of nervous syncytia is in the late fetal stage and it reaches maturity in the 2nd month.

Capillary veins appear in the late fetal stage and show no tendency to form capillary networks until the 1st month. In the 1st month, capillary veins increase and the networks are more dense than those in adults. However the density of capillary distribution in the 2nd month decreases and they come to resemble those in adults. The nervous syncytia make networks earlier than the capillary veins until the 1st month. The development of capillary veins does not correspond with that of nerve fibers.

3) The development of a nerve supply for the wall of the intestine.

The 1st order of intramural distribution of nerve fibers (REISER and STOEHR, jr.) has already developed in the late fetal stage. The 2nd and 3rd order begin to appear in the new-born and gradually differentiate to form complicated networks. In the 2nd month the nerve fibers are almost identical with those in adults. AUERBACH's plexus has already appeared in the late fetal stage, but MEISSNER's plexus is found first on the 15th day after birth. The outline of the nerve cells in these plexuses is not clear in the fetus, and accessory cells are very few and indistinct. In the 1st month, nerve cells are relatively clearly outlined and accessory cells surrounding nerve cells increase. They become mature in the 2nd month. As a whole, the neurofibrills of the 1st, 2nd and 3rd order, have a tendency to occur

earlier than the nerve cells.

4) Appearance of the myelin sheaths

Myelin sheaths cannot be found by EHRLICH's method in either the fetus or the new-born. They appear first on the 15th day after birth.

The development of the peripheral nervous system seems to be incomplete until the lst month after birth. It gradually comes to maturity in two months.

V. CONCLUSION

1) The nerve supply to the hair follicles in the ear appears first on the 15th day after birth, and concentrated ramifications of nerve fibers are observed in the 2nd month.

2) Nervous syncytia (JABONERO) are found first in the late fetal stage, and become mature in the 2nd month. In the fetus, SCHWANN's cells or interstitial cells (CAJAL) are not clearly observed, though their nuclei appear indistinctly already in the middle fetal stage.

3) Capillary veins are found already in the late fetal stage, but their networks cannot be found till the lst month. The development of nervous syncytia is rather earlier than that of capillary networks.

4) Nerve plexuses and nerve cells in the wall of the intestine appear already in the late fetal stage. They become mature in the 2nd month.

5) Myelin sheaths cannot be found in fetus and new-born. They appear first on the 15th day after birth.

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Explanation of figures

Fig. 1-6. (in the early fetal stage)

1) Thick bundles of nerve fibers. 3. 5 × 10.
2) Spinal nerves. 3.5 × 10.
3) Fine nerve fibers. 42 × 10.
4) The prevertebral ganglion. 10 × 10.
5) The prevertebral ganglion. 42 × 10.
6) Fine nerve fibers and a primordial tissue of large blood vessels. 42 × 10.

Fig. 7-10. (in the middle fetal stage)

7) Thick nerve fibers in the ear. 3.5 × 10.
8) Networks of fine nerve fibers in the ear. 95 × 10.
9) The immature nuclei of Schwann's cells. 95 × 10.
10) The prevertebral ganglion. 95 × 10.

Fig. 11-16. (in the late fetal stage)

11) Distribution of nerve fibers in the connective tissue of the ear. 3.5 × 10.
12) Fine nerve fibers in spaces between the primordium of hair follicles of the ear. 42 × 10.
13) Few immature nuclei of Schwann's cells. 95 × 10.
14) Auerbach's plexus in the wall of the small intestine. 95 × 10.
15) Networks of nerve fibers in the muscle layer of the small intestine. (The lst order according to REISER and STOEHR jr.) 42 × 10.
16) Capillary veins and nerve fibers. 42 × 10.

Fig. 17-24. (New-born)

17) Wavelike bundles of nerve fibers in the root of the ear. 10 × 10.

18) Fine nerve fibers running toward the hair follicles. 42×10.
19) Nervous syncytium. 95×10.
20) The nuclei of Schwann's cells. 95×10.
21) and
22) The lst order and 2nd order of intramural nerve plexuses in the wall of the small intestine. 10×10 (21) and 42×10 (22).
23) Fine nerve fibers in the submucous tissue of the small intestine. 95×10.
24) Nerve cells in the intramural nerve plexuses of the small intestine. 95×10.

Fig. 25-33. (Rabbits on the 15th day after birth)

25) and
26) Nerve fibers in the submucous tissue of the ear. 42×10 (both).
27) Nerve fibers of the hair follicles. 42×10.
28) Nervous syncytium. 95×10.
29) The myelin sheath. 42×10.
30) The intramural distribution of nerve fibers in the small intestine. 42×10.
31) Nerve cells of the intramural nerve plexuses of the small intestine. 95×10.
32) Meissner's plexus. 95×10.
33) Nervous syncytium in the submucous tissue of the small intestine. 95×10.

Fig. 34-39. (One month old rabbits)

34) and
35) Nervous syncytia. 95×10 (both).
36) Capillary networks in the subcutaneous tissue of the ear. 95×10.
37) Auerbach's plexus. 95×10.
38) Meissner's plexus. 42×10.
39) Intramural nerve fibers of the small intestine. 42×10.

Fig. 40-45. (Two months old rabbits)

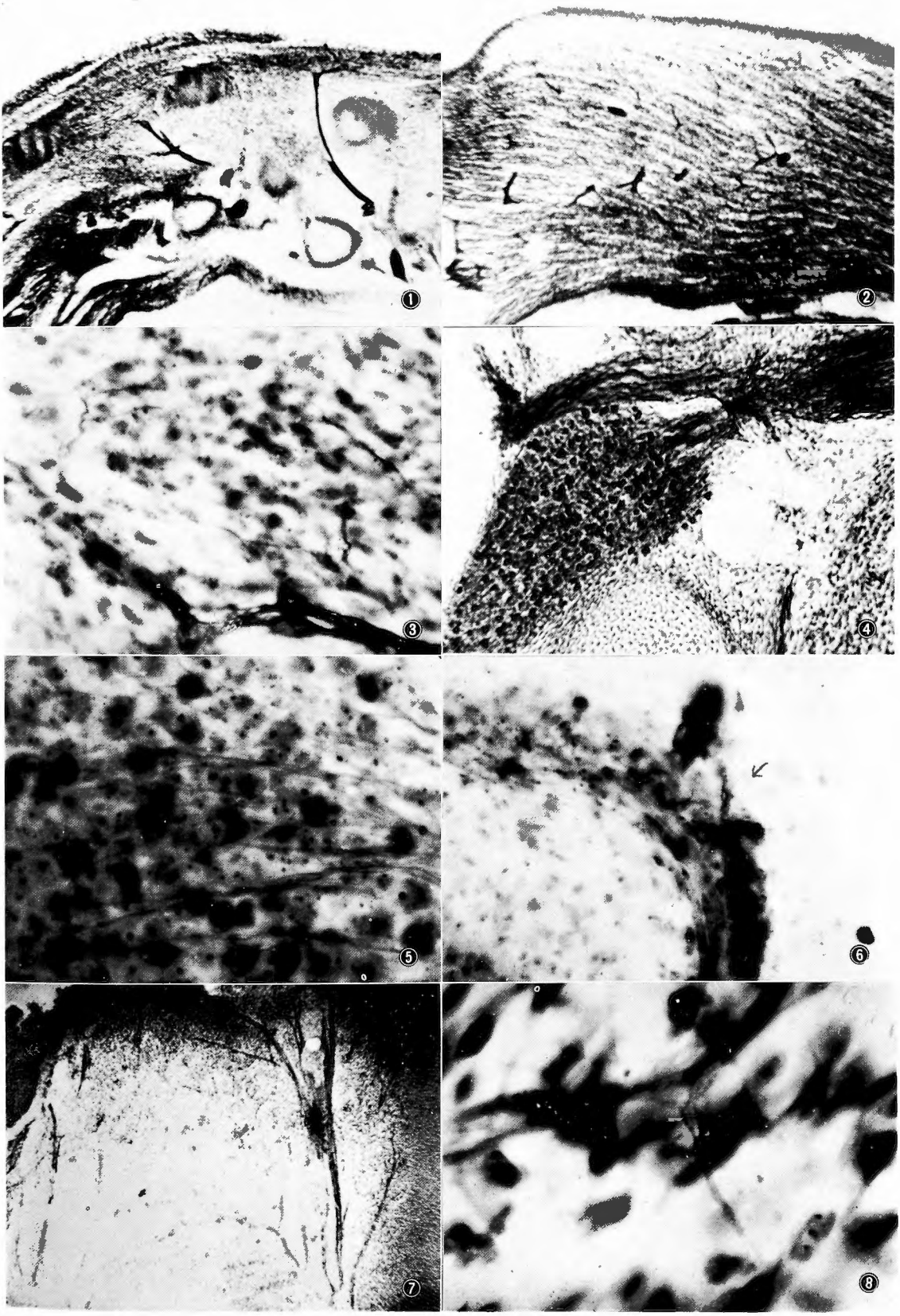
40) The concentrated ramifications of nerve fibers in the hair follicle. 42×10.
41) Capillary networks. 42×10.
42) Nervous syncytium in the subcutaneous tissue of the ear. 95×10.
43) Nervous syncytium in the submucous tissue of the bronchus. 95×10.
44) Capillary veins and intramural distribution of nerve fibers in the muscle layer of the small intestine. 42×10.
45) Nerve cells of Meissner's plexus. 95×10.

Fig. 46-48. (Three months old rabbits)

46) Nervous syncytium in the subcutaneous tissue of the ear. 95×10.
47) Meissner's plexus. 95×10.
48) Neurofibrillar distribution with nerve cells in the wall of the intestine. 42×10.

REFERENCES

1) Feyrter, F.: Ueber die Pathologie d. vegetativen nervoesen Peripherie u. ihrer ganglionaeren Regulationsstaetten. Wien, 1951.
2) Jabonero, V.: Der anatomische Aufbau des peripheren neurovegetativen Systems. Acta neuroveget., Wien, Supplementum **IV**., 1953.
3) Kruecke, W.: Die Entwicklung der peripheren Nerven. Handbuch d. speziell. Path. Anat. u. Hist., Ⅷ[-], Springer, 10. 1955.
4) Kuntz, A.: The Evolution of the Sympathetic Nervous System in Vertebrates. J. of Comparat. Neurology, **21**, 215, 1911.
5) Kimura, Ch.: Surgery of the Autonomic Nervous System(in Japanease), J. Jap. Surg. Soc., **52**, 450, 1951. The Problem of Abdominal Pain. Arch. Jap. Chir. **22**, 59, 1953. Physiology of Abdominal Pain (in Japanease). Rinsho no Shinpo, **7**, 131, 1953. Visceral Sensory Nerves and Autonomic Nerves. Saishin Igaku. **9**, 5, 34, 1954.
6) Kure, T. and Okinaka, S.: The Autonomic Nervous System (in Japanease). 1949.
7) Otsu, A.: A Histological Study of Sensory Nerve Endings in the Alimentary Canal of Human Beings and Dog. Acta Sch. Med. Univ. Kyoto, **31**, 103, 1953.
8) Riegele, L.: Beitrag zur Kenntniss des Scheidenplasmodium im autonomen Nervensytem.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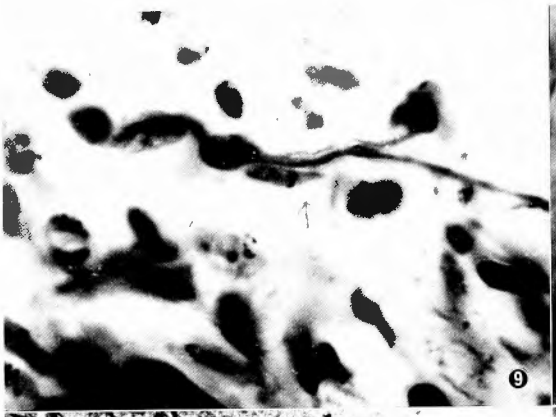
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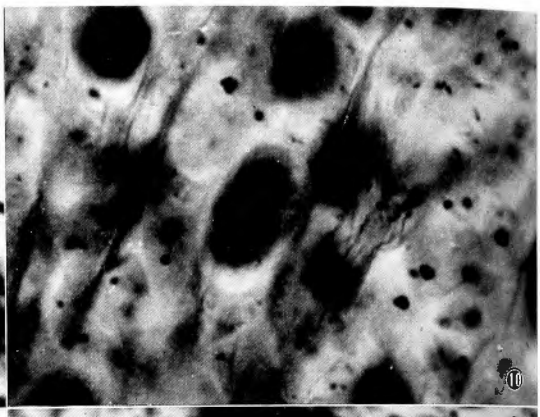
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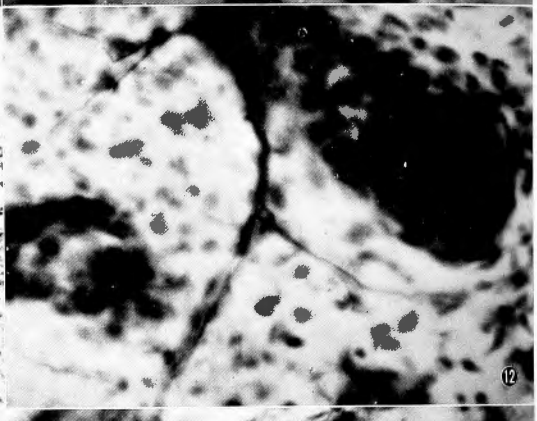
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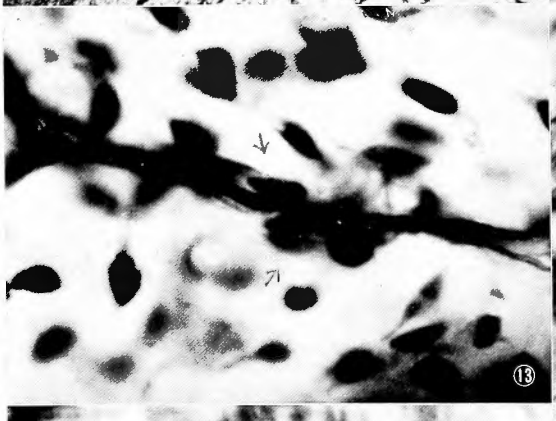
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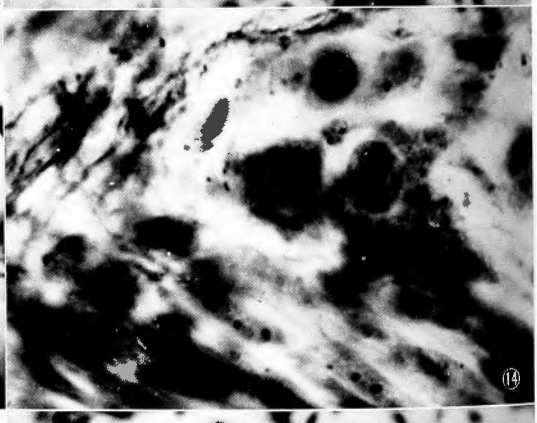
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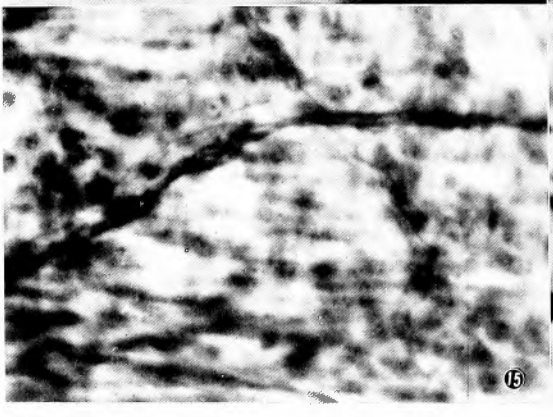
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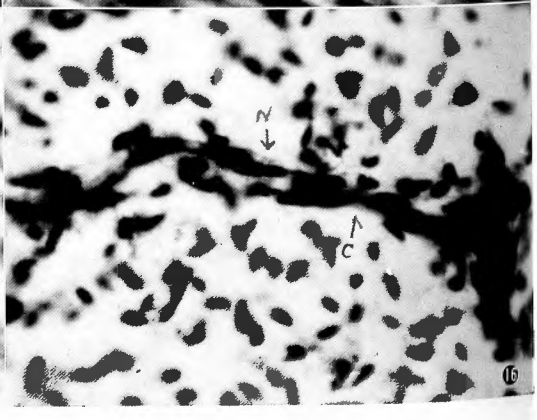

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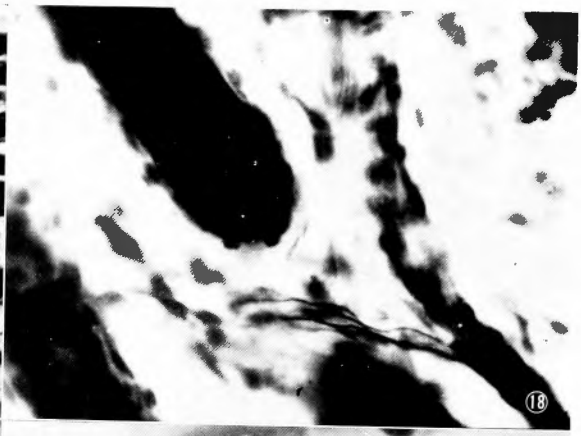
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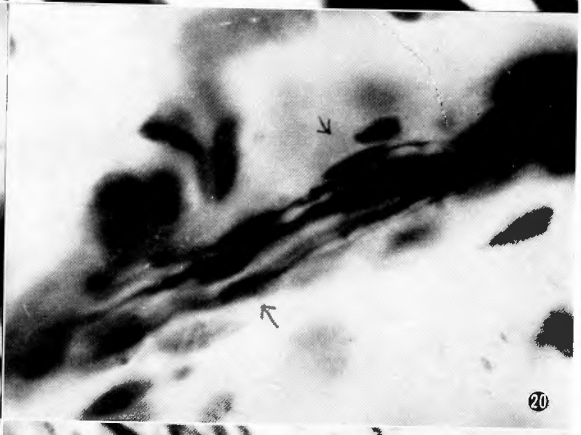
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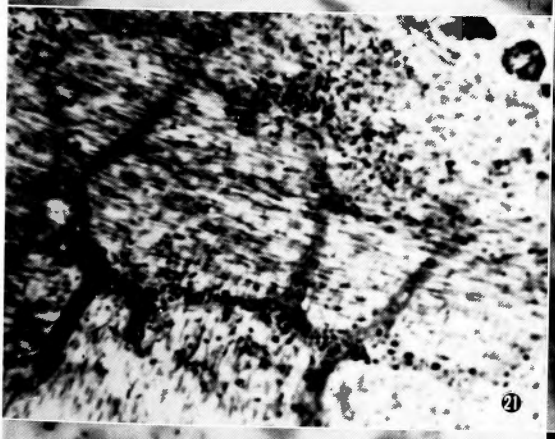
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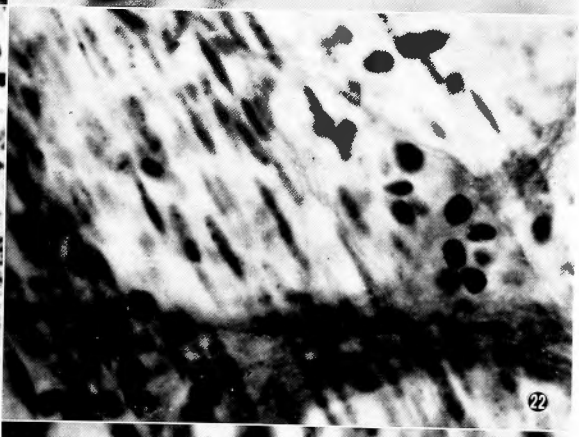
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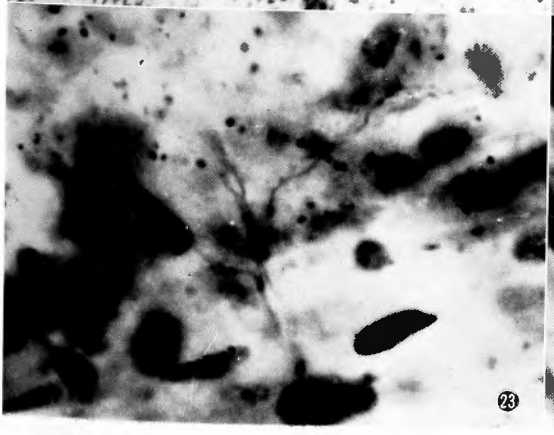
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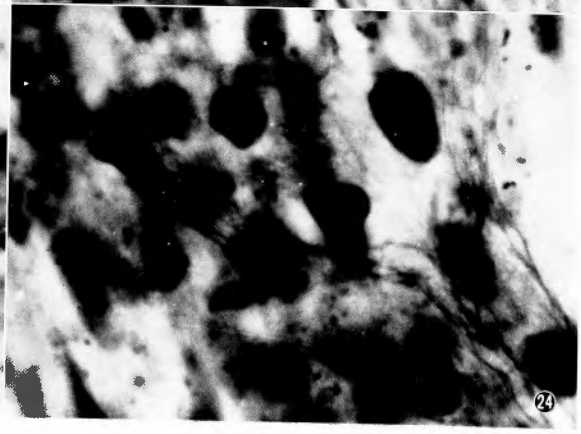
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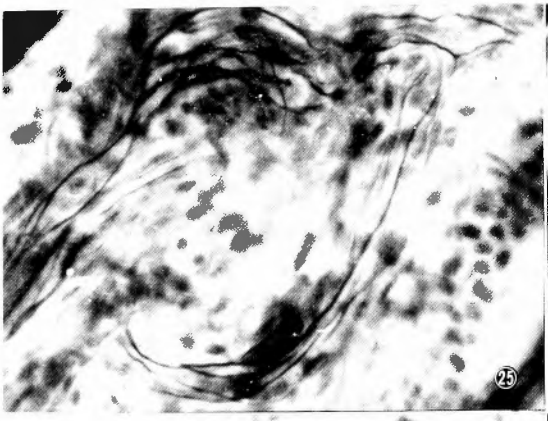
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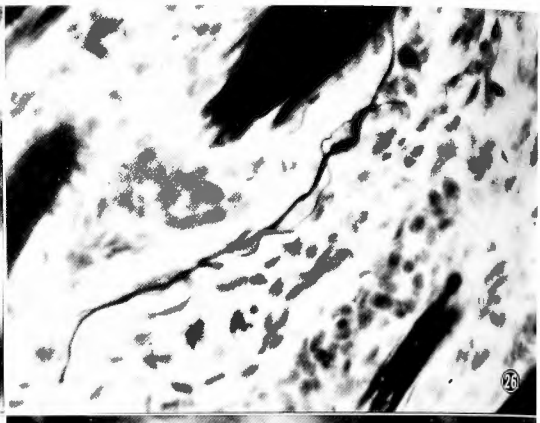
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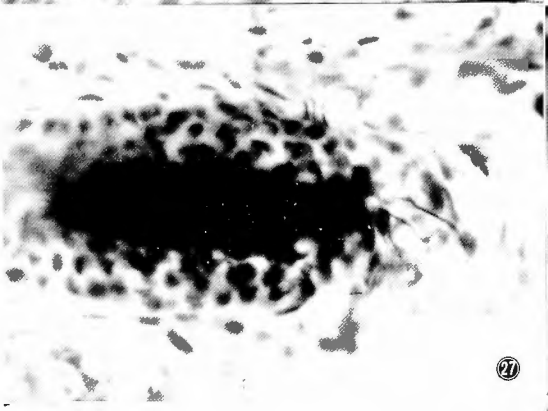
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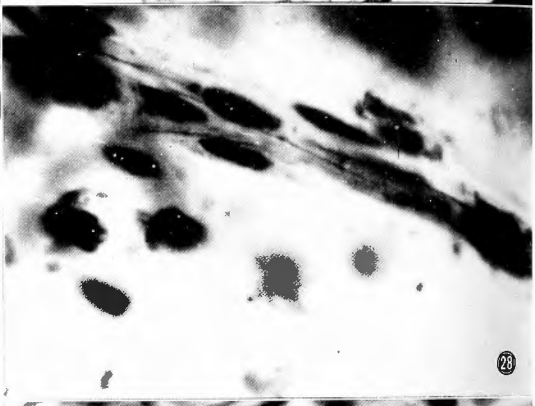
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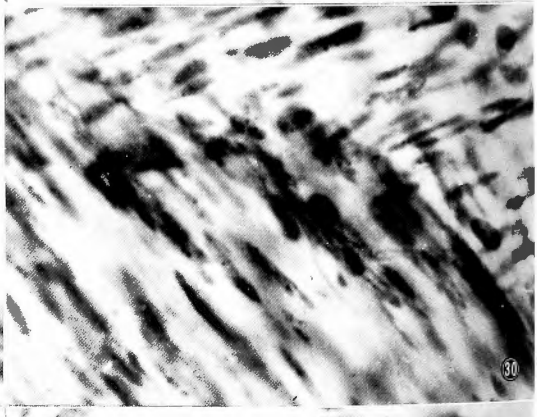
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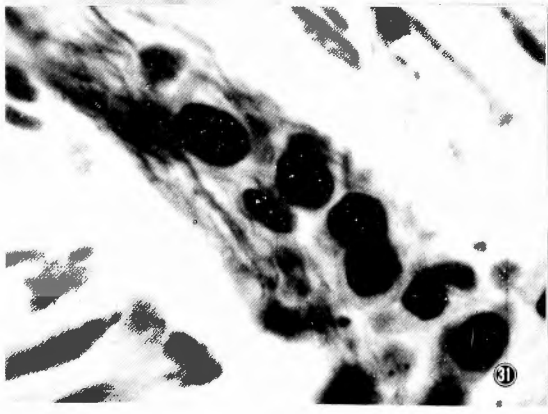
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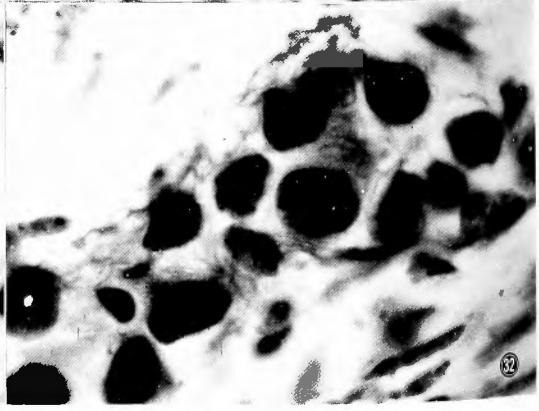
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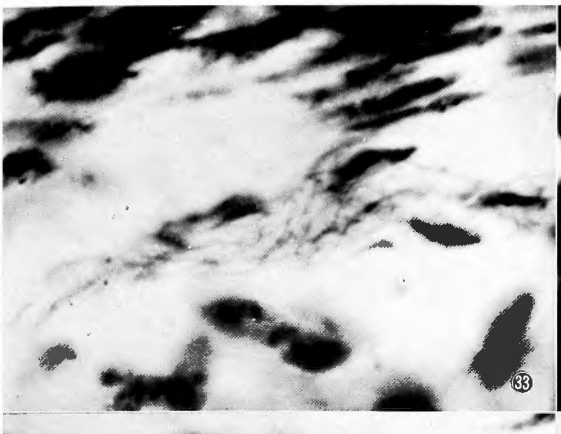
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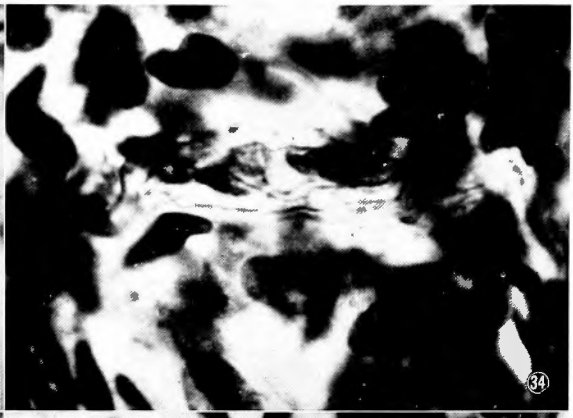
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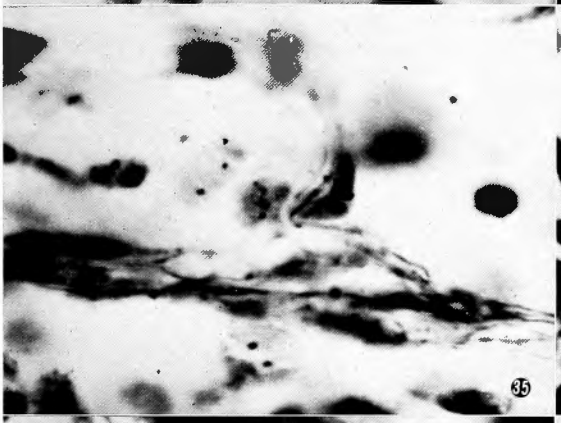
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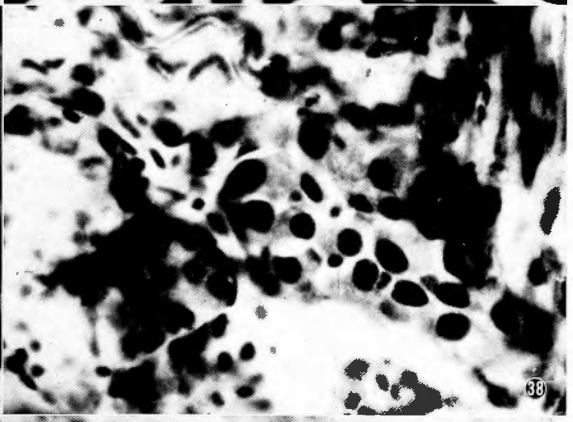
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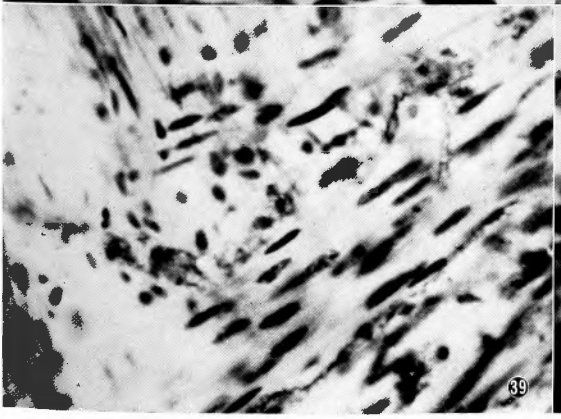
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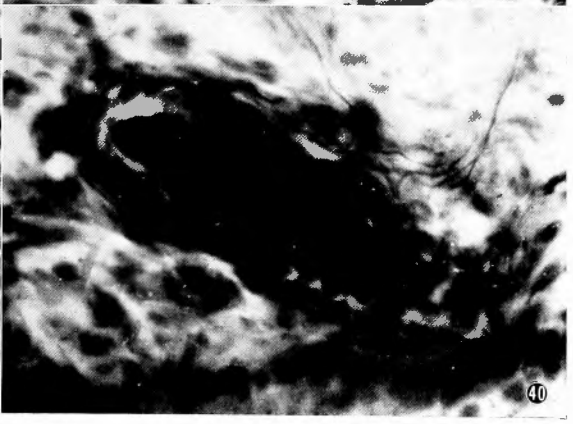
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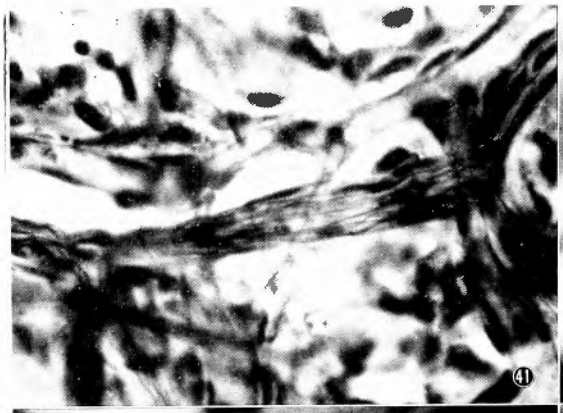
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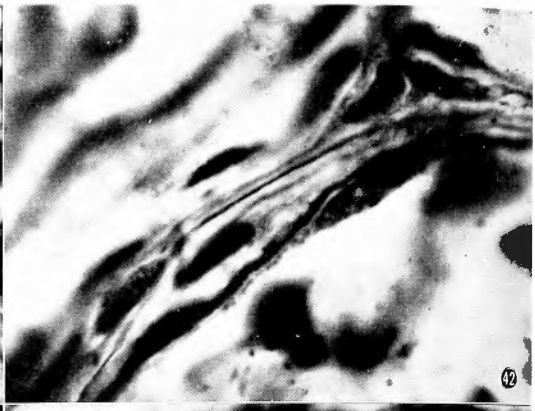
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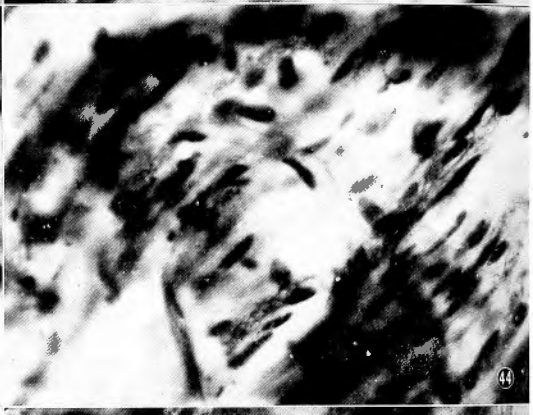
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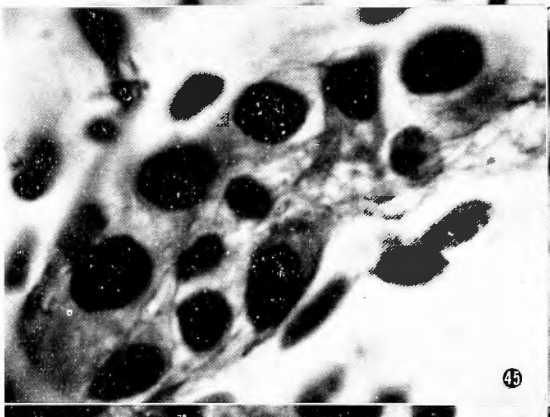
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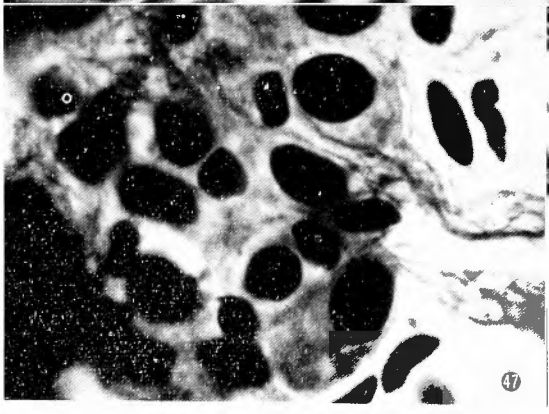
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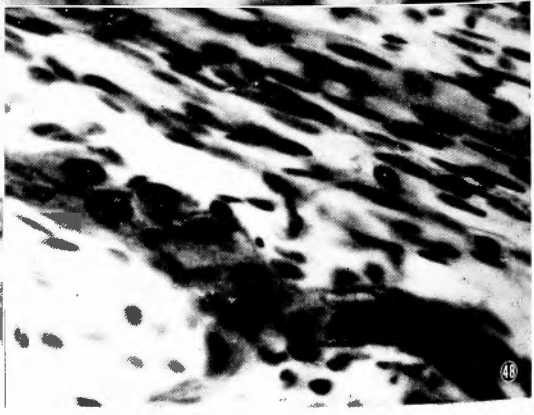
48

Z. f. Zellforsch. u. mikro. Anat., 15, 1932.
9) Seto, H.: Histological Observation of Human Sensibility. Rinsho no Nihon **1**, 32, 1955. (in Japanease) Studies on the Sensory Innervation (in Japanease). Igaku Shoin, 1957.
10) Stoehr, jr.: Ueber "Nebenzellen" und deren Innervation in Ganglien des vegetative Nervennsystems, zugleich ein Beitrag zur Synapsenfrage. Z. f. Zellforschung, **29**, 569, 1939. Intramurales Nervensystem des Rumpfdarmes, Nervengewebe, Lehrbuch d. Histologie u. d. mikroskop. Anatomie, 363, 1951.
11) Sunderplasmann, P.: Sympathikuschirurgie. Stuttgart, 1953.
12) Tsuzaki, T.: Embryology of Human Beings (in Japanease). Nihon Isho Shuppan, 1953.

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末梢神経系の発達過程に関する組織学的研究

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神経系の発生学的研究は多数報告されているが，その大部分は極めて初期の胎児の中枢神経系或は植物神経系原基の発生を研究したものであり，末梢神経系の発達過程に関しては，僅かに髄鞘の発達に就いて若干の報告を見るのみで，尚未知の点が多い．

著者は家兎を 1) 胎生初期， 2) 胎生中期， 3) 胎生後期， 4) 新生児， 5) 生後15日， 6) 生後1ヵ月 7) 生後2ヵ月， 8) 生後3ヵ月， 9) 成熟家兎の9群に分類し，その生長過程に於ける末梢神経（特に終末）の組織学的所見を種々の点で観察し次の如き知見を得た．尚，神経染色法としては Jabonero 氏法を髄鞘染色法としては Ehrlich 氏酸ヘマトキシリン法を用いた．

1) 毛根部（耳翼）への集中的神経分枝は生後15日に初まり生後2ヵ月で殆ど完成される．胎生後期及び新生児に於ては未発達の毛根原基の間を走る細い神経線維の枝をみるのみである．

2) 胎生中期に於て細い神経線維が僅かに網状を示し初めるが， Schwann 氏細胞は未だ不明瞭である．胎生後期に初めて Schwann 氏細胞の核と思われるものが認められる．神経線維の網状分枝とその細胞成分が生長と共に増加し複雑となり生後2ヵ月には成熟家兎と殆ど同様な nervous syncytium を示す．即ち nervous syncytium は胎生後期で出現し初め生後2ヵ月で略完成する．これに対し毛細血管網は nervous syncytium の発達より遅れ，生後1ヵ月で初めて出現し，この時は非常に緻密であるが，生後2ヵ月にはその密度を減じ成熟家兎と同様な分布を示す．

3) 小腸壁筋層に於ける神経線維分布の 1st order は胎生後期に於て既に認められ，2nd 及び 3rd order は新生児から認められる．これらの線維は徐々に増加し分枝し，生後2ヵ月では成熟家兎のそれと殆ど同様の分布を示す．Auerbach 神経叢は胎生後期， Meissner 神経叢は生後15日から認められる．これらの神経叢中の神経細胞及び特に Nebenzelle (accessory cell) は生後2ヵ月には成熟家兎のそれと殆ど同様となる．全体的にみて腸壁筋層ではその神経叢の細胞成分の発達は神経線維の分化発達に較べて稍遅れる傾向にある．

4) 髄鞘は新生児では未だ認められず生後15日に初めて認められる．

以上を総括すれば，組織学的にみて，家兎の末梢神経系は生後1ヵ月迄は不完全であり，生後2ヵ月にして殆ど成熟家兎の状態に達する．